CRITICAL CARE

Micro-fragmented fat injection reduces sepsis-induced acute inflammatory response in a mouse model

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Abstract

Background: Severe sepsis has a high mortality rate. There is increasing evidence that human mesenchymal stem cells possess immunomodulatory properties in sepsis, particularly those from adipose tissue. We hypothesised that micro-fragmented human fat, obtained with minimal alteration of the stromal vascular niche, attenuates the inflammatory response and improves outcome in a murine model of sepsis.

Methods: Micro-fragmented fat, lipoaspirate, or saline was administered intraperitoneally 2 h after caecal ligation and puncture (CLP) in C57Bl/6RJ ketamine—xylazine anaesthetised mice. The primary endpoint was the inflammatory score. Secondary endpoints included survival, physiological, histological, and biological parameters.

Results: In CLP mice, micro-fragmented fat administration significantly decreased the median (range) inflammatory score compared with saline [17 (14—20) *vs* 9 (8—12), P=0.006]. Secondary endpoints were also significantly improved in micro-fragmented fat-treated compared with saline-treated CLP mice. Improvement in inflammatory score and in survival was suppressed when micro-fragmented fat was co-administered with liposomes loaded with clodronate (macrophage toxin) or NS-398 (cyclo-oxygenase 2 inhibitor), but not with SC-560 (cyclo-oxygenase 1 inhibitor).

Conclusions: In a murine model of severe sepsis, micro-fragmented fat improved early inflammatory status and outcome, at least in part, by a cyclo-oxygenase-2-mediated mechanism. The potential therapeutic value of micro-fragmented fat in severe sepsis warrants further investigation.

Keywords: human adipose stem cells; inflammation; mesenchymal stem cell; prostaglandin—endoperoxide synthases; sepsis

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> **Editor's key points**
> - Stem cells from adipose tissue have anti-inflammatory properties.
> - Human micro-fragmented fat from liposuction. Decreased inflammatory score and increased survival in a mouse model of sepsis.
> - The mechanism may involve cyclo-oxygenase.
> - Micro-fragmented fat warrants further study as a treatment for sepsis.

Severe sepsis is characterised by circulatory, cellular, and metabolic abnormalities associated with a systemic uncontrolled inflammatory response (cytokine storm) leading to vascular leakage, tissue damage, multi-organ failure, and a high mortality rate.[1] Of the innovative therapeutics relevant to severe sepsis, human mesenchymal stem cells (MSCs) from adipose tissue (human adipose stem cells; hASCs) are promising.[2] Several lines of evidence indicate that these cells exhibit organ-protective properties and improve survival rate in sepsis models.[3–12] Interestingly, hASCs have been shown to induce the activation of macrophages which possess potent immunomodulatory ability and therapeutic potential in sepsis.[8,9] This macrophage activation was shown to occur via the cyclo-oxygenase (COX)-2/prostaglandin pathway in septic patients.[13] hASCs may therefore help control the inflammatory response at the acute phase of severe sepsis and improve outcome. hASCs are routinely obtained enzymatically from fat lipoaspirate (LA) as stromal vascular fraction (SVF), and, in most cases, undergo further *ex vivo* expansion. In addition to the high cost, the large number of processing steps makes its use difficult in an emergency context. Lipogems® (LG) is an innovative and commercially available system that *in vivo* provides micro-fragmented fat in a short time (about 20 min), without expansion or enzymatic treatment. With the aid of this technology, fat is gently micro-fragmented and washed to remove oil and blood residues.[14] To date, its immunomodulatory properties have not been investigated in the context of severe sepsis.

We hypothesised that LG administered shortly after the onset of the inflammatory process attenuates organ injury and improves outcome in mice with severe sepsis. We also hypothesised that the cyclo-oxygenase 2 (COX2)/prostaglandin pathway is involved. Inflammatory score (IS) 24 h after the induction of sepsis was used as the primary endpoint of the study along with survival, body temperature, histological and biological parameters, and plasma cytokine levels as secondary endpoints. The possible cellular mechanisms involved in the immunomodulatory effects of LG were also investigated.

Methods

Animals

All protocols were reviewed and approved by the Institut Pasteur Ethics Committee and approved by the Institut Pasteur, the competent authority, for compliance with French and European regulations on Animal Welfare and with Public Health Service recommendations. Six-to ten-week-old C57Bl/6RJ male mice were obtained from Jackson Laboratories and were allowed to habituate for 1 week before experimentation. They were housed on a 12:12 light/dark cycle in a pathogen-free facility with controlled temperature and humidity. Based on our previous experience, in which we observed that 24—48 h after caecal ligation and puncture (CLP), 100% of mice died when reaching an IS of 18—20, mice with an IS between 18 and 20 were monitored with greater attention and mice with an IS of 21 or higher were culled and considered dead for welfare reasons.[15,16] Mice were killed by cervical dislocation without anaesthesia. To ascertain anaesthesia, the absence of oculo-palpebral reflex (corneal reflex) and the interdigital toe pinch reflex was performed (approved by the Institut Pasteur internal ethics committee). The animal procedures concurred with the EU Directive 2010/63/EU and are reported in accordance with the ARRIVE guidelines.[17] The total number of mice included in this study was 70.

Patients

Each patient signed an informed consent of no objection for the use for research of surgical tissues (otherwise destined for destruction). Patient data were collected on a prospectively held database setup after the approval of the French National Authority for Personal Data Protection (Commission Nationale Informatique et Liberté: CNIL. https://www.cnil.fr, Registration number 1922081—02.02.2016).

Sepsis model and anaesthesia

CLP resulted in peritonitis as reported elsewhere.[18] Briefly, animals were anaesthetised with ketamine (Imalgene 1000, 100 mg kg^{-1}; Merial) and Xylazine (Rompun 2%, 20 mg kg^{-1}; Bayer) i.p. before surgery. Mice were rehydrated with 1 ml of 0.9% NaCl at 37°C twice a day for 48 h and treated with buprenorphine (0.3 mg kg^{-1}; Axience) twice a day for 2 days after surgery. After laparotomy, the caecum was ligatured at the distal third with a 4.0 suture, and the distal part was perforated twice with a 21 gauge needle. Perforation of the caecum, triggering the release of faecal material into the peritoneal cavity, was performed.

Harvesting of human adipose tissue

For a complete description of the process, see Supplementary material and methods. Four healthy women aged 36, 56, 49,

and 41 yr underwent elective fat harvesting for aesthetic morphological procedures, under general anaesthesia. The lower or the lateral abdomen gluteal and thigh regions were chosen as donor sites for fat harvesting. Before harvesting the fat, the site was injected with carbocaine and adrenaline at very high dilution (2% and 1 mg ml^{-1}, respectively) using a disposable 17G blunt cannula connected to a 60 ml BD Luer-Lok™ Syringe (Becton Dickinson, Franklin Lakes, NJ 07417, USA). The fat was then harvested using a 13G blunt cannula, for a fast and a-traumatic suction, connected to a Vaclock® 20-ml syringe.

Processing of the adipose tissue

The lipoaspirate (LA) was immediately processed in the Lipogems® processing kit, a disposable device that progressively and gently reduces the size of the adipose tissue clusters with a mild mechanical action while eliminating oily substances and blood residues which may have pro-inflammatory properties. The entire process, carried out in one surgical step, was performed under complete immersion in physiological solution minimising any trauma to the cell products. The resulting micro-fragmented fat was collected in a 60 ml syringe, positioned for decanting the excess saline solution. The final product (LG) was transferred into several 1 ml syringes to be reinjected i.p. in the recipient mouse within 2 h. Each LG from human subjects was injected in three to four mice (CLP and controls), and the LG samples were not pooled.

To test whether the stroma was key in the anti-inflammatory properties of LG, we extensively chopped it with small sterile scissors until a bulk of adipose tissue was obtained. When enzymatically processed, LG was digested with 0.1% collagenase (Sigma-Aldrich, St Louis, MO, USA) at 37°C for 20 min with gentle agitation. After extensive washing [resulting bulk washed with 50 ml sterile phosphate buffered saline (PBS) and centrifuged at 500g 3 times], 150 µl of the resulting bulk was injected i.p. into CLP mice 2 h after the induction of sepsis and the effects were compared with those of intact LG.

To obtain Lipogems®-derived ASCs (LG-ASCs), LG was plated in Mesencult proliferation kit for human MSCs (StemCell Technologies Inc.). The cells were cultured for 1 week at 37°C with 5% CO_2 and were then washed and detached with trypsin (1 mM for 2 min at room temperature). Overall, 93% of the cells were CD44$^+$/CD73$^+$/CD90$^+$ by cytometry confirming the purity of the population.[19]

Macrophage elimination

To determine the role of macrophages in the effects of LG in septic mice, we attempted to eliminate macrophages *in vivo* by injecting liposomes loaded with clodronate (a macrophage toxin, 20 µl g^{-1} from a 5 mg ml^{-1} stock of clodronate liposomes). Mice were injected i.p. every 12 h from 2 days before CLP either with clodronate liposomes (20 µl g^{-1}) from a 5 mg ml^{-1} stock solution or saline. After culling, the spleen was sampled, chopped, filtered through a 40 µm filter, and stained with anti-mouse F4/80 AlexaFluor 488 (eBiosciences; clone BM8) to quantify the number of macrophages by cytometry.

Cylco-oxygenase experiments

CLP mice were injected with indomethacin (a non-selective inhibitor of COX enzymes, 10 mg kg^{-1}; Sigma-Aldrich, St. Louis, MO, USA; $n=8$), or SC-560 (a selective inhibitor of COX1, 6 mg kg^{-1}; Sigma-Aldrich, $n=8$) or NS-398 (a selective inhibitor of COX2, 6 mg kg^{-1}; Sigma-Aldrich, $n=8$) i.p. at the time of LG injection. Injections were performed every 8 h for 24 h. All injections were body weight corrected, starting from a fresh 10 mg ml^{-1} solution before injections with 0.9% NaCl. The control group consisted of CLP mice injected with the same volumes of 0.9% NaCl only.

Multiplex immunoassay

Cardiac puncture was undertaken in anaesthetised animals to collect blood into heparin coated Eppendorf tubes and syringes. The blood was centrifuged at 500 x g at 4°C for 10 min and the plasma frozen for subsequent analysis using Luminex® multiple cytokine and chemokine analysis (Bio-Plex® ProTM Mouse Cytokine Standard 23-Plex, Group I and Standard 9-Plex, Group II).

Biological parameters and white blood cell count

Blood chemistry measurements were undertaken on a Koné lab 20 (Thermo Scientific) using an adapted kit for: aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK) activities (bioMérieux) and creatinine (Jaffé method; Fisher Brahms). For white cell count, blood was directly added to a scil Vet abc haematology analyser calibrated and set up for mouse parameters.

Histochemistry and immunohistochemistry

Liver and kidneys were fixed in 4% formaldehyde for 2 days and embedded in paraffin at 60°C. Tissues were then sectioned (50 µm) and dried overnight and stained with haematoxylin−eosin. Standard immunostaining techniques were used to prepare frozen serial 10 µm sections, fixed with paraformaldehyde and incubated with 0.5% TritonX100 and 3% bovine serum albumin in PBS. After extensive washing, sections were incubated with primary antibodies at 37°C for 1 h, washed with PBS, and then exposed to the secondary antibodies (Histofine, simple stain Max PO peroxidase polymer directed against the correct host) for 50 min at room temperature. Negative controls without the primary antibody were performed concurrently. The count was done manually and by an investigator blinded to treatment groups. The antibodies are detailed in the supplementary information.

Outcome measures

The Murine Sepsis Score described by Shrum and colleagues[20] was used 24 h after CLP as the primary endpoint: appearance, level of consciousness, activity, eyes, respiration rate, and respiration quality was scored from 0 to 4 (see Supplementary Table S1).

Secondary endpoints were survival until day 60 and body temperature after CLP until day 21. Mice with an IS higher than 21 were considered dead. Biomarkers of hepatic failure (AST, ALT, ALP) and biomarkers of systemic inflammation (LDH, CK, leucocyte count, plasma levels of pro- and anti-inflammatory cytokines) were recorded at 24 h and until day 21. In addition, the numbers of F4/80$^+$, B220$^+$, and CD3$^+$ cells in the liver and kidney 6 h after CLP were collected.

Data and statistical analysis

The studies were conducted in a randomised blinded fashion to minimise bias. CLP mice were ear-tagged with random numbers (Harvard Apparatus) for allocation to experimental group. In preliminary experiments, we observed better survival with low variability in the group of mice injected with bone marrow-derived MSCs (BMSCs) during sepsis. Indeed, 65% of animals died after sepsis against 5% when injected with BMCs. Based on these effects, we anticipated that the expected difference in the IS 24 h after induction of sepsis between CLP with and without LG should be in the same range as BMSCs[21]. With an alpha risk of 5% and a power of 90%, the number of animals per group needed to be at least 7. Data are expressed as median, inter-quartile, and full range, except for body temperature cytokine levels and leucocyte count, which are presented as mean (SD). Anonymisation was maintained until just before statistical analyses were carried out (Fig. 1).

Statistical analysis was undertaken using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA) using non-parametric Kruskal–Wallis and *post hoc* testing with Dunn's correction for multiple comparisons, or Mann–Whitney *U*-test as appropriate. For survival experiments, a non-parametric log-rank test (Mantel–Cox test) was used, and for comparison of IS and body temperature over time, a two-way analysis of variance (ANOVA) with Tukey's multiple comparisons test was performed. A minimum of 95% confidence interval was set for significant *P* values, indicated in figures as <0.05 (*), <0.01 (†), and <0.001 (‡). All animals were included in the analysis. All experiments were repeated once independently with two different sets of LG and LA each with seven or eight mice per group.

Results

Effects of micro-fragmented fat on inflammatory response in septic mice

The cellular composition of LG was evaluated and analysed using cytometry. Results are described in Supplementary Table S1. No statistically significant differences in terms of cellular composition were found between donors or between LG and LA.

Primary endpoint

At 24 h after induction of sepsis, a significant decrease in IS between control (CTR) and LG mice [17 (14–20) *vs* 9 (8–12); P=0.006] was detected (Fig. 2a). The IS remained high (~16) in CTR mice for 5 days and decreased markedly between days 7 and 9. The IS was 7 (5–9) at day 21 in CLP and 3 (2–5) in LG mice (P=0.03; Supplementary Fig. S1a). When injecting LA from the same patient at the same time, no difference in IS was observed
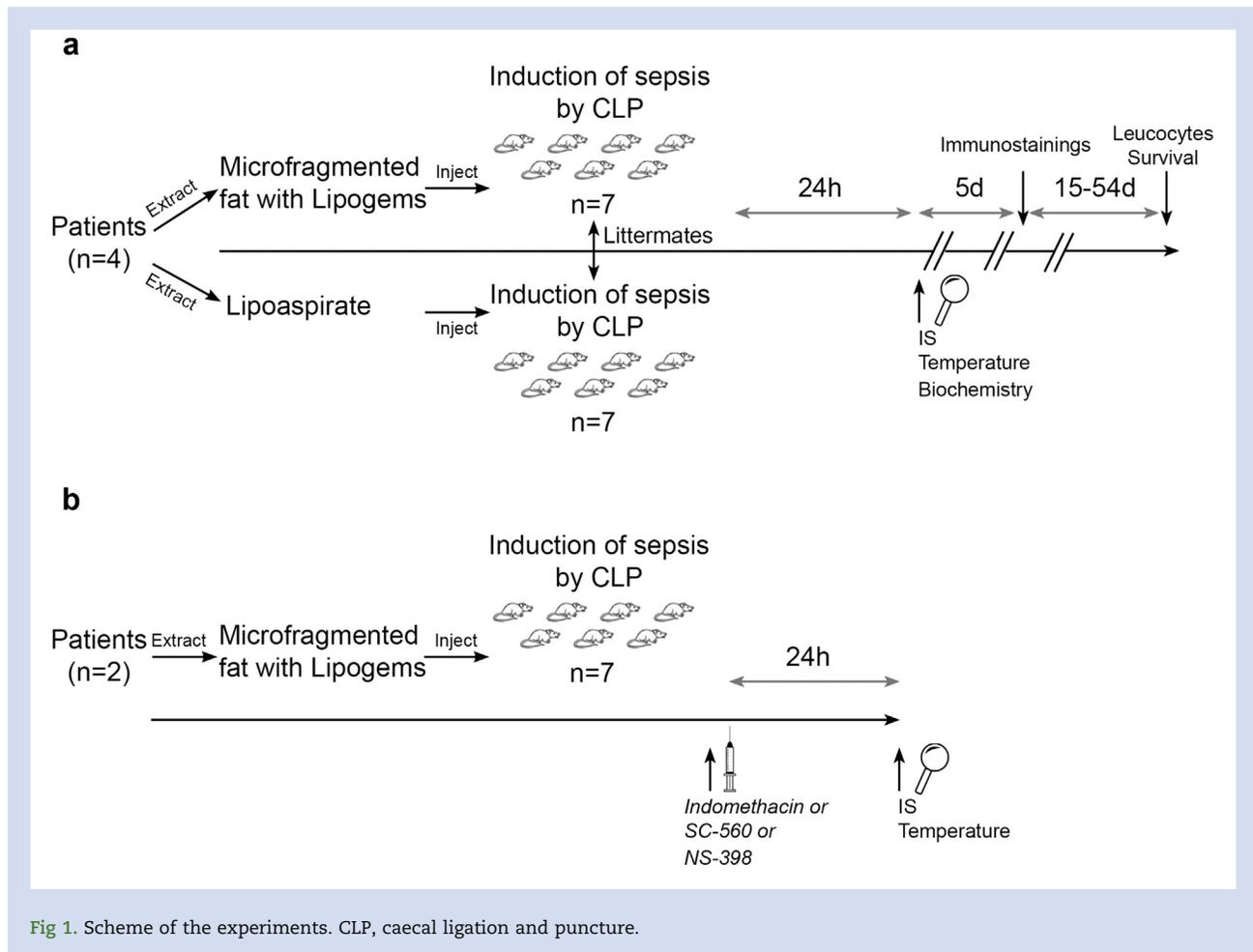
Fig 1. Scheme of the experiments. CLP, caecal ligation and puncture.
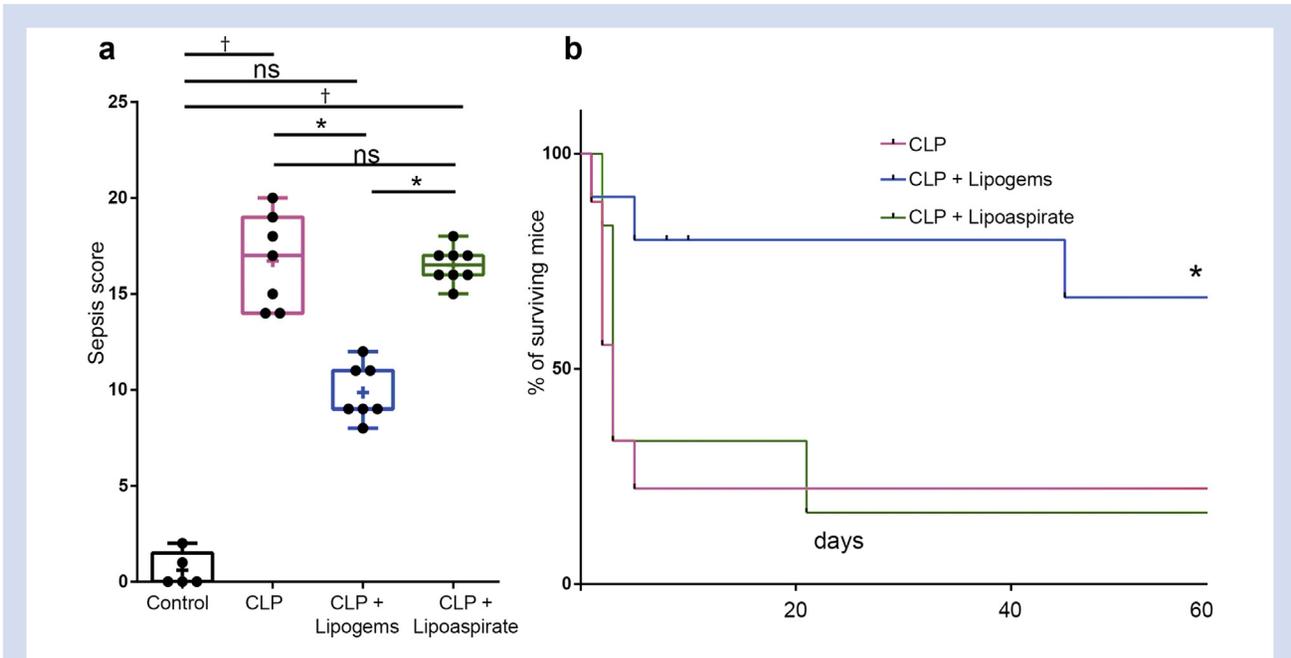
Fig 2. (a) Inflammatory score 24 h after induction of CLP of control (*n*=5), CLP-CTR (*n*=8), CLP-LG injected mice (*n*=8), and CLP-LA (*n*=8). (b) Survival curves of septic (CLP-CTR) and septic and Lipogems® (CLP-LG) or septic and lipoaspirate (CLP-LA) injected mice (C57Bl/6RJ; *n*=8 per time point). Data are shown as individual data points with median, IQR and full range *P<0.05; †P<0.01. CLP, caecal lication and puncture; CTR, control; LG, Lipogems®; LA, lipoaspirate; ns, non-significant.

at 24 h [IS=16.5 (15−18); P=0.9, Fig. 2a]. No differences in IS were observed between different donors of fat (Supplementary Fig. S1e). In addition, 24 h after the injection of LPS, the IS was significantly lower in *LPS*-LG mice compared with untreated *LPS* mice or *LPS*-LA mice (Supplementary Fig. S2a).

Secondary endpoints

Survival. A significant improvement in survival rate of LG mice compared with CTR mice was found (Fig. 2b), but injection of LA did not improve survival (Fig. 2b). In LPS and *LPS*-LA mice, 71% were alive at 48 h, but by day 4 all animals had died. In *LPS*-LG, the survival rate was 100% at 48 h and 71% at day 4 (Supplementary Fig. S2b).

Body temperature. LG mice had higher temperatures and a smaller decrease in body temperature compared with CTR mice (Supplementary Fig. S2c). The difference in body temperature held until day 14. At day 19, the body temperature of both groups was at normal range (Supplementary Fig. S2c). LA injection did not affect body temperature at any time.

Biological data. Plasma enzyme activities were higher at 24 h in CTR mice compared with *control* groups (Fig. 3a) but were similar to control in LG mice (Fig. 3a) and had a smaller initial decrease in leucocytes at 24 h; Fig. 3b). No difference in leucocyte counts was detected between groups at late time points (Supplementary Fig. S3a).

Cytokine plasma levels. Plasma cytokine levels were elevated 24 h after the induction of sepsis (Fig. 4a−d and Supplementary Table S2). Levels of key pro-inflammatory cytokine were significantly lower in LG mice compared with CTR mice (Fig. 4a and b and Supplementary Table S2). Conversely, plasma levels of anti-inflammatory cytokines (IL-4, G-GCF) were higher in LG mice compared with CTR mice (Fig. 4c).

Immune cell organ infiltration. Higher in numbers of F4/80+ (macrophages), B220+ (B lymphocytes), and CD3+ (T lymphocytes) cells were seen in the liver of inflammatory CLP mice at 6 days, compared with non-septic mice (Fig. 5a, Supplementary Fig. S4a,b). After LG injection, significantly fewer F4/80 cells were observed compared with CTR mice (Fig. 5a). There was also a global decrease in all immune cell types tested in the kidney after LG injection (Fig. 5b, Supplementary Fig. S4c,d). We decided to study immune organ cell infiltration on the 6th day after CLP as no cell infiltration was detected in those organs at 24 h, 2 days and 4 days after CLP.

Protective action of micro-fragmented fat

Separate Lipogems® components are ineffective in reducing sepsis-related inflammatory response

In CLP mice treated with chopped or enzymatically digested LG, no abrogation of IS was observed at 24 h (Supplementary Fig. S1b). Likewise, neither had the injection of the supernatant or LA any effect on IS (Supplementary Fig. S1d). Finally, LG mice had a lower IS at 24 h compared with LG-ASCs (Supplementary Fig. S1c).

LG mice had a lower IS at 24 h compared with BMSC mice (Supplementary Fig. S1c).

Mechanisms of protective effects of micro-fragmented fat

Host macrophages play a determinant role in the protective effect of micro-fragmented fat

After injecting LG mice with liposomes loaded with clodronate, we observed a decrease in the number of F4/80 cells in
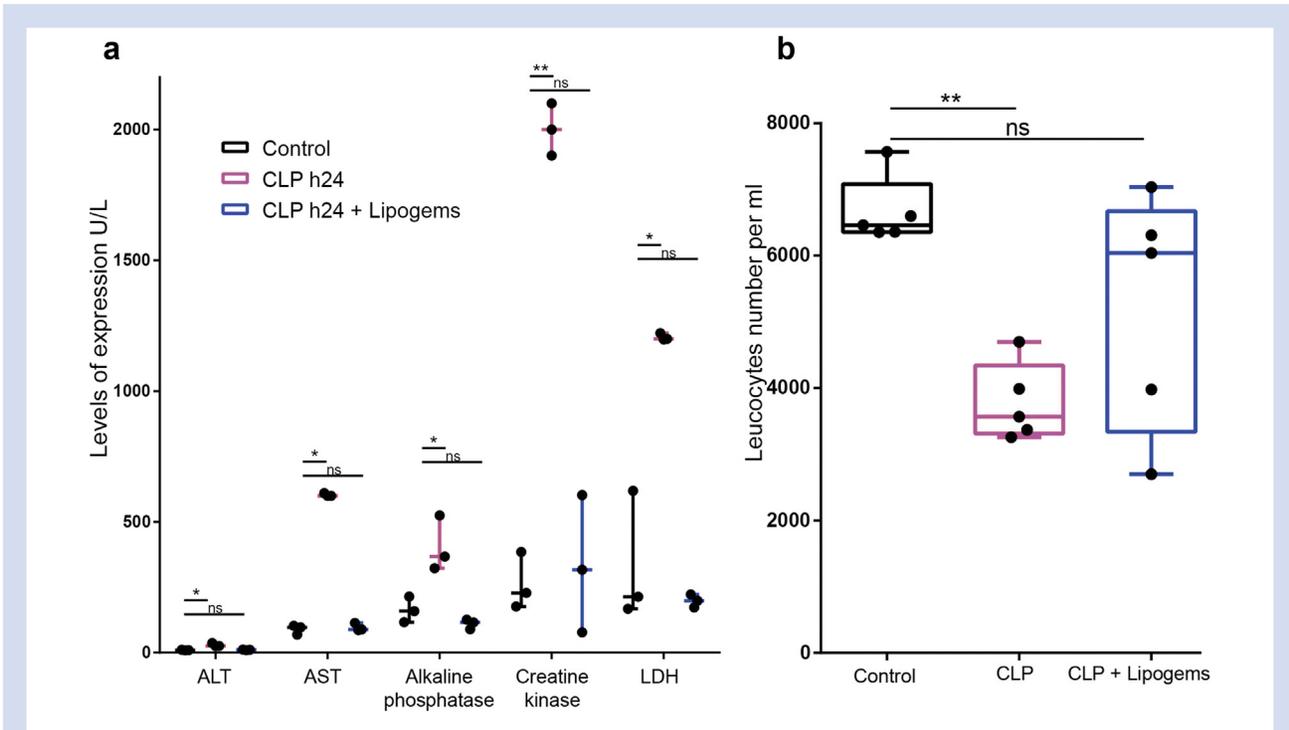
Fig 3. (a) Alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase, creatine kinase, and lactate dehydrogenase (LDH) in control (*n*=4), CLP-CTR (*n*=4), and CLP-LG mice (*n*=5). (b) Leucocyte count after blood sampling 24 h after induction of CLP in control (*n*=5), CLP-CTR (*n*=5), and CLP-LG mice (*n*=5). Data are shown as individual data points with median, IQR, and full range. *P<0.05; [†]P<0.01. CLP, caecal lication and puncture; CTR, control; IQR, inter-quartile range; LG, Lipogems®; LA, lipoaspirate; ns, non-significant.

the spleen (Supplementary Fig. S2d,e), associated with the abolition of the effects noted after LG administration on IS (Supplementary Fig. S2f).

Micro-fragmented fat exerts an anti-inflammatory effect through the cyclo-oxygenase 2 (COX-2)/PGE2 pathway

When LG mice were co-treated with indomethacin or NS-398, the IS was similar to that of CTR mice but was lower when LG was co-injected with SC-560 (Fig. 6a). These results were reflected in the effects on plasma cytokine levels (Fig. 6b, Supplementary Table S3).

Discussion

The main findings of the present study can be summarised as follows: intact human micro-fragmented fat (Lipogems® - LG) markedly improved the inflammatory score and survival rate in a murine model of severe sepsis. This effect may be mediated, at least in part, *via* COX-2, and LG may offer a novel potential treatment for further investigation. Severe sepsis is characterised by circulatory, cellular, and metabolic abnormalities associated with a systemic uncontrolled inflammatory response leading to vascular leakage, tissue damage, multi-organ failure, and a high mortality rate.[1] Current treatments are not very effective.[22] Patients who survive sepsis frequently suffer from muscle wasting and continued loss of muscle proteins resulting in muscle atrophy and weakness with significant clinical consequences.[23,24] These detrimental effects are partially

attributable to the uncontrolled initial cytokine storm culminating in damage to tissues and organs.

MSCs from adipose tissue are routinely obtained enzymatically from fat lipoaspirate as SVF, followed by further *ex vivo* expansion. The large number of processing steps makes the use in an emergency context difficult. Thus, a minimally manipulated adipose tissue product that preserves the naturally occurring ASC content would have remarkable clinical relevance. Lipogems® is an innovative technique that intraoperatively provides micro-fragmented fat in a short time, without expansion and/or enzymatic treatment. Using this technology, fat can be micro-fragmented and washed from pro-inflammatory oil and blood residues, while preserving viable elements with perivascular identity within an intact stromal vascular niche.[21]

To examine the effects of LG on the inflammatory response and survival, we used a murine model of severe inflammation: peritonitis induced by CLP. LG injection induced a lower IS 24 h after the induction of sepsis. Noteworthy, the low IS remained stable between days 1 and 21, suggesting a long-lasting anti-inflammatory effect. Survival rate, body temperature, cytokine plasma levels, leucocyte/macrophages organ infiltration, and blood leucocyte count were also improved. In line with previous findings of direct inhibition of macrophage migration by LG,[25] the observed decrease in pro-inflammatory cytokines induced by LG was associated with a marked reduction (30—50%) of immune infiltration (macrophages and lymphocytes in the liver and kidney), which is clinically relevant.[26—28] This organ protective effect is possibly mediated by the MSC population within LG[29—32] which possess anti-inflammatory
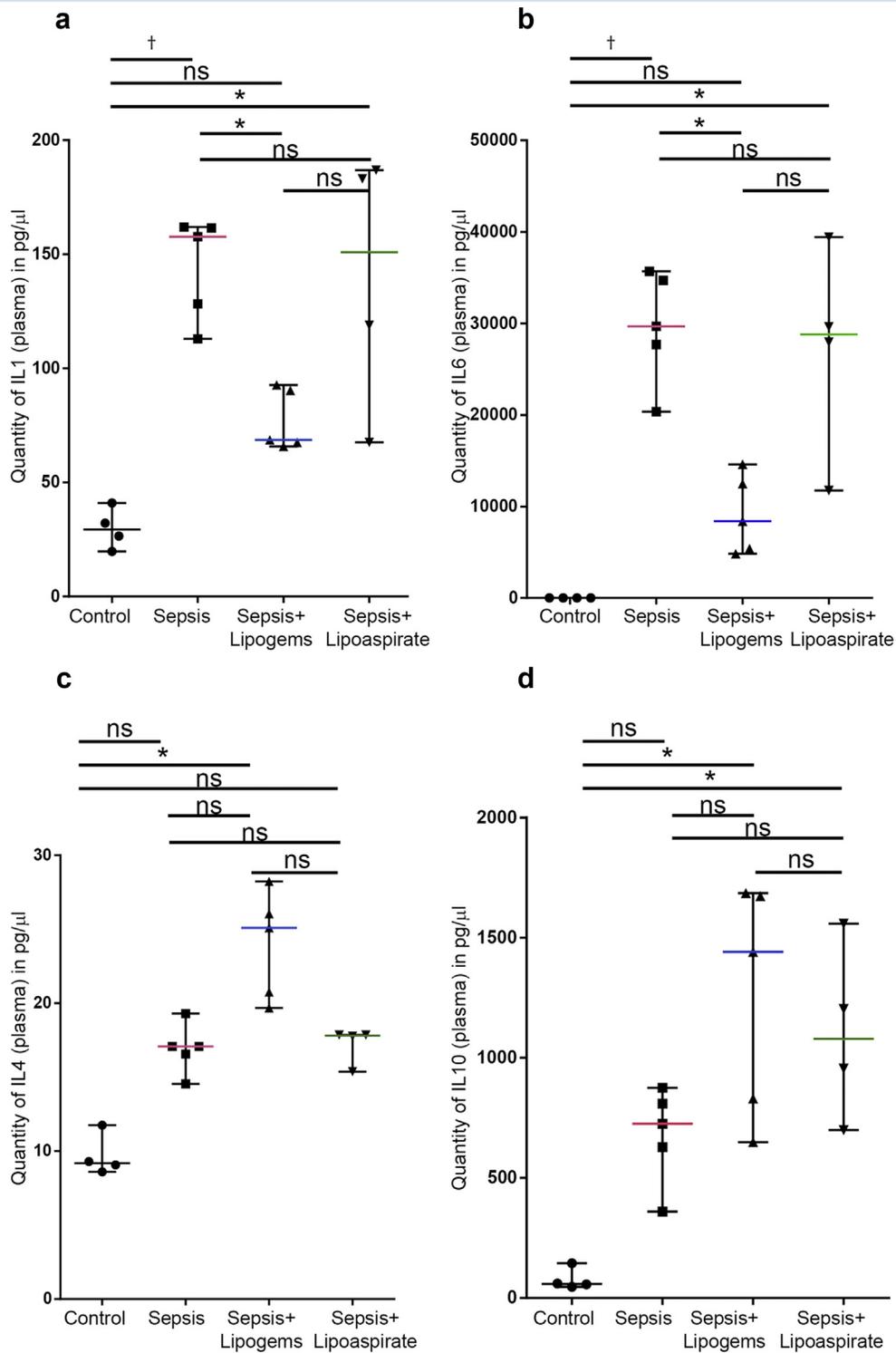
Fig 4. Plasma (a) IL-1, (b) IL-6, (c) IL-4, and (d) IL-10 levels: no CLP ($n{=}4$); CLP 24 h (CLP-CTR $n{=}5$); CLP 24 h + Lipogems® injected mice (CLP-LG $n{=}5$); and CLP 24 h + Lipoaspirate injected mice (CLP-LA $n{=}4$); assessed using Luminex (multiplex assay). Data are shown as individual data points with median and full range. *P<0.05; †P<0.01; ‡P<0.001. CLP, caecal lication and puncture; CTR, control; IL, interleukin; IQR, inter-quartile range; LG, Lipogems®; LA, lipoaspirate; ns, non-significant.

Fig 5. Number of F4/80+ cells in (a) liver and (b) kidney in C57Bl/6RJ control mice (no CLP, no Lipogems injection *n*=6), CLP (CLP-CTR *n*=7), and CLP + Lipogems® injected mice (CLP-LG *n*=7). Data are shown as individual data points with median, IQR, and full range. *P<0.05; [†]P<0.01; [‡]P<0.001. Liver and kidney came from the same mice. CLP, caecal lication and puncture; CTR, control; IQR, inter-quartile range; LG, Lipogems®; ns, non-significant.
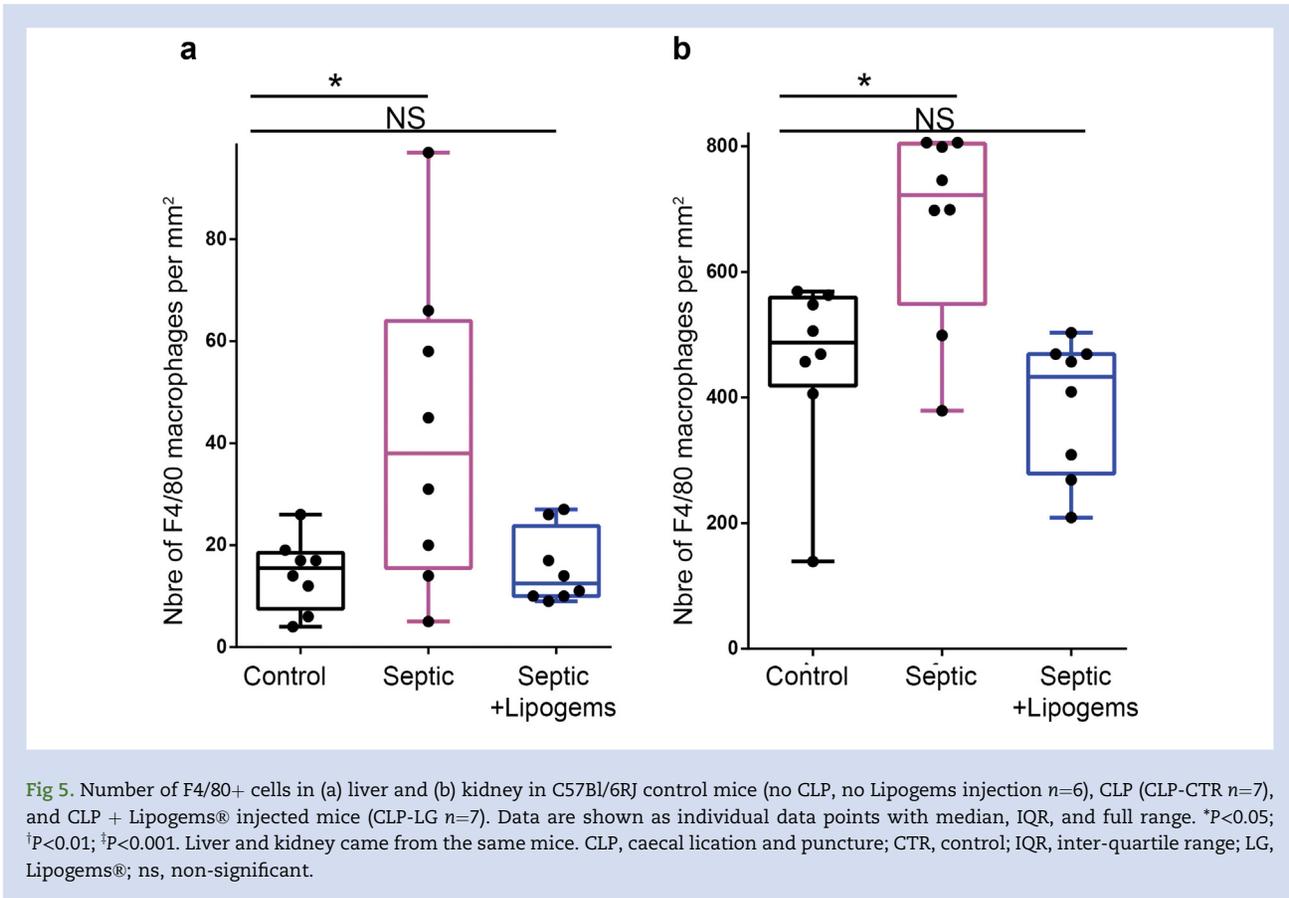
and protective properties[29,33] if the stromal vascular niche is intact.[14] This may account for the more potent effect of LG in decreasing the IS compared with (1) isolated and/or cultivated MSCs in which the manipulation processes may trigger senescence and decline in their immunomodulatory properties[34] and with (2) mechanically or enzymatically disrupted LG and LA. Indeed, we found that the ability of both digested LG and LA to decrease the IS at 24 h was less than that of intact LG. This emphasises the critical role of the stem cell niche,[35] a structure defined as the microenvironment in which the adult stem cells reside surrounded by immune and epithelial cells. Our findings suggest a key role of the Lipogems® processing method in triggering new biological properties in the lipoaspirate. The main structural and morphological adipose unit, the adipose niche, is likely to be maintained after the processing and protects the non-activated native MSCs, allowing them to become activated when needed, strengthening their effectiveness in the recipient environment.

Prostaglandins are important mediators and modulators of the inflammatory response to infection and sepsis,[36–38] and COX inhibition has been reported to improve sepsis survival in 72% of previous reports.[39] Furthermore it has been shown that MSCs act on macrophages *via* the prostaglandin pathway.[40] For this reason, we investigated the role of COX inhibition and the role of LG in secretion of prostaglandin E_2 which converts macrophages (among other inflammatory cells) to the phenotype that releases the anti-inflammatory cytokines.[41] We showed that the immunomodulatory action of LG may be mediated, at least in part by COX-2, although other

mechanisms and pathways may also be involved. Our results indicate that activity of COX-2 (but not COX-1), which is inducible during inflammation in many cell types,[37,38] is critical for the anti-inflammatory effect of LG. At the doses used here, SC-560 and NS-398 primarily affect prostaglandin synthesis, and non-COX targets are unlikely to be involved.[37] Although indomethacin has been shown to improve sepsis in some models, it was detrimental in others.[41,42] Such discrepancies may be the result of timing and/or dose and the sepsis model. Injection of the inhibitors alone neither improved nor worsened the inflammatory response in our hands such that it is unlikely that the effects were a result of the action on the septic status, although this cannot be ruled out. LG alone inhibited inflammation caused by sepsis, and this effect was abrogated by co-treatment with the COX2 inhibitor NS-398 or the less specific COX inhibitor indomethacin, suggesting that COX2 activity is high in LG treated mice.

Our study has several limitations. First, although our main outcome measures IS and survival rate were at an early time point, outcomes at 48 h after CLP included only surviving mice. To avoid this bias, as many experiments as possible used a 24 h post-CLP time point when all mice were alive. As a result, surviving mice may have been less dramatically impacted by sepsis. Second, we used buprenorphine after the induction of sepsis for animal welfare reasons,[15] and we cannot exclude that the combination of buprenorphine and LG would impact on our observed effects although there was no evidence of this. Last, we know that MSCs can synthesise antibiotic proteins (LL37) that kill bacteria and stimulate macrophages.[5,43]
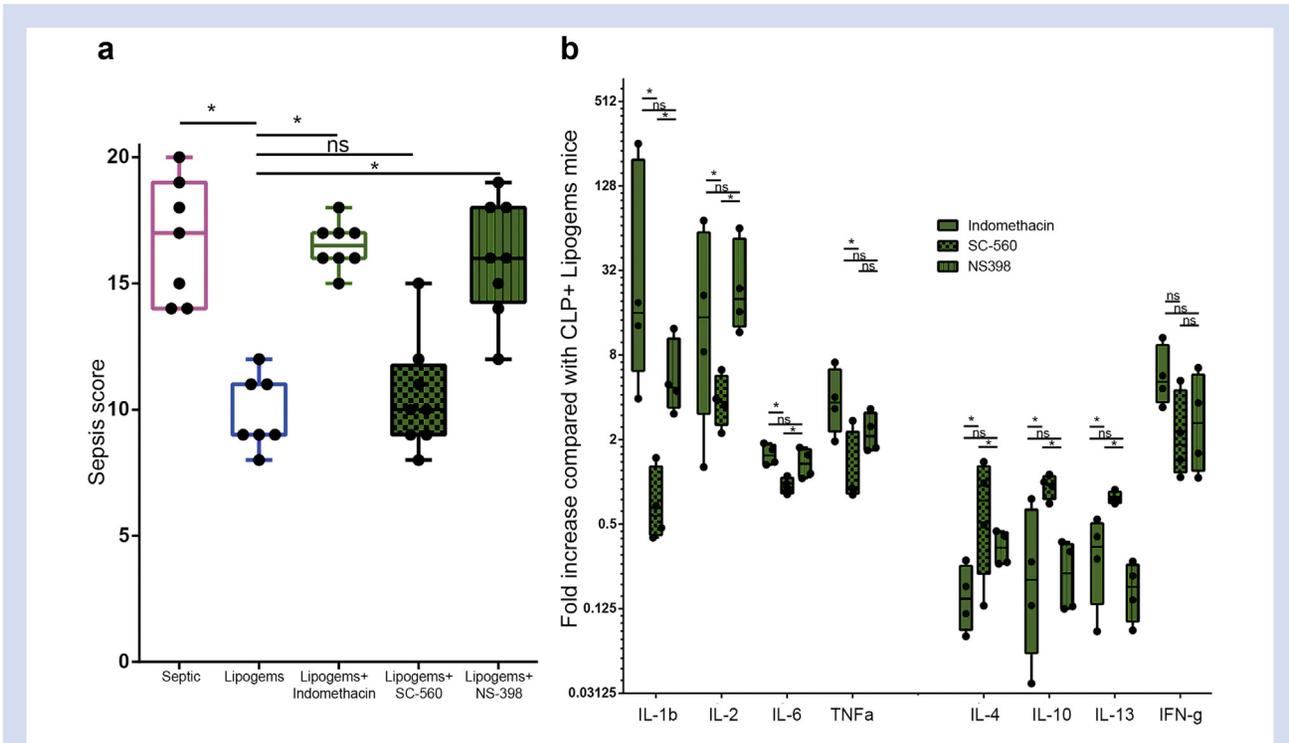
Fig 6. (a) Inflammatory score 24 h after induction of CLP in septic (CLP-CTR, $n=7$), CLP + Lipogems® (CLP-LG $n=7$), CLP + Lipogems® + indomethacin ($n=8$), CLP + Lipogems® + SC-560 ($n=8$), CLP + Lipogems® + NS-398 ($n=8$). (b) Fold increase of key plasma pro- and anti-inflammatory cytokines of septic (CLP-CTR, $n=5$), CLP + Lipogems® (CLP-LG $n=8$), CLP + Lipogems® + indomethacin ($n=8$), CLP + Lipogems® + SC-560 ($n=8$), and CLP + Lipogems® + NS-398 ($n=8$). Data are shown as individual data points with median, IQR, and full range. $P<0.05$; [†]$P<0.01$; [‡]$P<0.001$. CLP, caecal lication and puncture; CTR, control; IQR, inter-quartile range; LG, Lipogems®; ns, non-significant.

We did not investigate such effects but cannot exclude that micro-fragmented fat also directly participated in bacterial clearance.

In conclusion, we have shown that LG exerts potent immunomodulatory properties in experimental models of severe sepsis. Further examination of the potential of LG for clinical use in severe sepsis and the possible molecular mechanisms are now under investigation.

Authors' contributions

Design of laboratory experiments: A.B., P.R.
Conduct of experiments and data analysis: A.B., P.R., J.M.
Design of experiments: M.H., J.M., F.C.
Extraction of fat from patients during surgery: M.H.
Assistance in performing macrophage experiments by setting up the good timing and concentrations and test of different doses of COX inhibitors (pilot experiments): A.H.
Assistance in performing experiments and involvement when blinding (IS notably) was required: D.B.
Inventor of Lipogems® system and provided advice in designing experiments: C.T.
Writing of the manuscript: J.M., F.C.

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Declarations of interest

C.T. is the president of Lipogems and inventor of the Lipogems® system (ref patent). F.C. and A.C. are members of the scientific council of Lipogems Inc. All of the other authors declare that they have no conflict of interests.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bja.2018.03.032.

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